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TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
AREA 1: INDOOR ECOSYSTEM CHAMBERS	3
Materials and Methods	3
Results (Winter 1976-1977)	9
Discussion (Winter 1976-1977)	11
Results (Spring 1977, Summer 1977)	18
Discussion (Spring 1977, Summer 1977)	19
AREA 2: OUTDOOR ECOSYSTEM CHAMBERS	20
Materials and Methods	22
Results	24
Discussion	25
AREA 3: SURVIVAL STUDIES:	26
<i>Melosira mummuloides</i>	26
Materials and Methods	26
Results	29
Discussion	30
<i>Acartia clausi</i>	30
Materials and Methods	30
Results	32
Discussion	33
SUMMARY	33
LITERATURE CITED	35

ASSESSMENT OF THE IMPACT OF INCREASED SOLAR ULTRAVIOLET
RADIATION UPON MARINE ECOSYSTEMS

Space Shuttle operations through the stratosphere may lead to a small reduction of the earth's ozone layer, with a resultant increase in transmission of solar ultraviolet radiation in the 290-320 nm waveband (UV-B). Studies indicated under the Climatic Impact Assessment Program (CIAP) of the U. S. Department of Transportation demonstrate that simulated solar ultraviolet radiation can, under experimental conditions, detrimentally affect the marine organisms (algae and small invertebrates) that form the base of the food web of oceanic and estuarine ecosystems. These organisms survive in nature by a combination of mechanisms for tolerating the detrimental effects of UV-B radiation. The key question is whether a small increase in biologically harmful ultraviolet radiation might overwhelm these mechanisms and produce changes that will have damaging consequences to the biosphere.

Although UV-B radiation penetrates a relatively short distance into highly productive estuarine waters, Calkins (1975) has reported that, when the organic content of oceanic waters is low, a pathlength of nearly 15 meters may be required to reduce the UV-B radiation to the 10% level. The behavioral responses of marine organisms which tend to protect them from excessive exposure to biologically harmful ultraviolet radiation are cued, most likely, by long-wavelength UV and visible radiation, a waveband which would be unaffected by a partial depletion of stratospheric ozone. Therefore, marine forms which are capable of tolerating present levels of UV-B radiation would lack the cue for a protective response to enhanced levels of UV-B radiation. On the other hand, if organisms do respond to UV-B radiation by a downward migration in the water column, they would move into a region

of different ecological, chemical, and physical properties, including decreased photosynthetically active radiation. Thus, there is a potential for an adverse effect of increased UV-B radiation in either case.

The objective of the present study is to provide data to assess the potential impact upon marine ecosystems if Space Shuttle operations contribute to a reduction of the stratospheric ozone layer.

Specifically, the study has addressed the following two questions:

1. Is there potential for irreversible damage to the productivity, structure and/or functioning of a model estuarine ecosystem by increased UV-B radiation or are these ecosystems highly stable or amenable to adaptive change?

2. What is the sensitivity of key community components (the primary producers, consumers, and decomposers) to increased UV-B radiation?

Three areas of study were examined during the past year:

1. A continuation of the study utilizing the two seminatural ecosystem chambers located at the Oregon State University Marine Science Center, Newport, Oregon.

2. A pilot study utilizing three flow-through ecosystem tanks enclosed in a small, outdoor greenhouse located in Newport, Oregon.

3. Sensitivity studies of representative primary producers and consumers.

AREA 1

MATERIALS AND METHODS

Two seminatural ecosystem chambers of a continuous flow-through design were employed for three studies (Winter 1976-1977, Spring 1977, and Summer 1977). These units were located at the Oregon State University Marine Science Center, Newport, Oregon (44°38'N). Each chamber had a capacity of 720 liters. The bottom of each chamber was graduated in a "stair-step" manner to provide a series of seven steps ranging in depth from 10 cm to 58 cm (Fig. 1). Seawater, drawn from the lower Yaquina Estuary, flowed through each of the chambers at a rate of approximately six liters per minute and provided for the natural recruitment of organisms into the chambers.

Visible radiation was supplied by a combination of "cool-white" fluorescent lamps and 60 W incandescent bulbs at a surface intensity of about 10,000 lumens/m² (Weston Illuminance Meter). Supplemental ultraviolet radiation was supplied by fluorescent sunlamps (Westinghouse FS40) filtered by a 0.13 mm thickness of presolarized cellulose triacetate film (Kodacel). The Kodacel acted as a 290 nm cut-off filter. To compensate for measured photodegradation, the filter was changed weekly.

For the Winter 1976-1977 study a stepped 24-hour photocycle was utilized with incandescent lights "on" from 0800 h to 2000 h, the "cool-white" fluorescent lamps "on" from 1000 h to 1800 h, and the other sunlamps "on" from 1100 h to 1700 h. The peak spectral irradiance in the 290-315 nm waveband at the surface of one chamber was 1.0 W/m² and at the other it was 1.4 W/m² (Fig. 2). The difference in intensity between the two chambers was created by the use of aluminum reflectors and different degrees of photodegradation of the Kodacel filters prior to use. The spectra at wavelengths

greater than 370 nm were similar. Typical local downward global irradiance at a solar zenith angle of 25° is 1.3 W/m^2 in the 290-315 nm waveband.

For the Spring 1977 and Summer 1977 studies a 24-hour photocycle was utilized with the "cool-white" fluorescent and incandescent lights "on" from 0600 h to 2000 h, and the sunlamps "on" from 0930 h to 1630 h. The peak spectral irradiance in the 290-315 nm waveband at the surface of one chamber was 0.50 W/m^2 and at the other it was 0.42 W/m^2 . A 0.25 mm thickness of the Kodacel filters was used in these studies.

Irradiance measurements in both the ultraviolet and visible range were made with a Gamma Scientific 2900SR Spectroradiometer system. The monochromator housing of this system interchangeably accommodates either a B & L ultraviolet grating assembly with approximately a 2 nm bandwidth in the 190-400 nm range or a B & L visible grating assembly with approximately a 4 nm bandwidth in the 350-800 nm range. This instrument has been modified and characterized by the U. S. National Bureau of Standards: A teflon diffuser has been substituted for the "fused silica" diffuser in order to give better cosine response and greater sensitivity in the UV-B region. The wavelength readability and short-term precision were improved to approximately 0.1 nm by adding a helipot dial for wavelength indications. Also, a solar-blind filter (Corion SB-1.25) was utilized during ultraviolet determinations in order to significantly reduce stray light problems inherent with the extended range photomultiplier detector. The NBS made a wavelength calibration, determined the spectral responsivity of the instrument in the region from 275 nm to 350 nm, and determined the slit function. Standard lamps traceable to the NBS were used in our laboratory to check irradiance measurements. The ultraviolet standard (Optronics Laboratories, Model UV-40) was calibrated from 180 nm to 400 nm and the visible standard (Gamma

Scientific, Model 220-9A) was calibrated from 380 nm to 1100 nm. Also, NBS supplied our laboratory with two BZS sunlamps (Westinghouse Electric) which had been calibrated from 275 nm to 350 nm.

The penetration of UV-B radiation through the water column of the two chambers was determined by utilizing a submersible CIAP model R-B Meter. Due to the proximity of the chamber walls to the UV detector, "apparent" absorption coefficients were calculated for comparative purposes. To obtain more accurate diffuse attenuation coefficients, a distance of at least two attenuation lengths is required between the wall of the experimental chamber and the radiation detector (R. Smith, personal communication). In our case, this would have ranged from 59 to 123 cm. This was not possible in our experimental chambers.

A series of samples of attached algae was collected during the three studies from assemblages that developed on acrylic plates located on the steps of the chambers. These were analyzed for concentration of photosynthetic pigments, community biomass, and community composition. The acrylic plates were cleaned and replaced after each set of samples was collected. Split-samples were utilized for the chlorophyll and biomass analyses, one-half of each sample being utilized for pigment extraction and the other half being used for biomass determination. Chlorophyll *a* concentrations were determined weekly by both the SCOR/UNESCO technique (Strickland and Parsons, 1972a) and the Moss-Lorenzen technique for determining chlorophyll *a* and phaeo-pigments (Strickland and Parsons, 1972b). The samples for both of these techniques were processed in subdued light. They were filtered through 4.5 cm Whatman GF/C glass filters which had been coated with magnesium carbonate. The samples were then homogenized in a Thomas, pestle-type grinder for 1-2 minutes at 500 rpm, extracted in the

dark in fresh 90% aqueous spectrophotometric grade acetone for 20 minutes with regular mixing, centrifuges in swing-out tubes, and measured immediately by spectrophotometer at 750, 663, 645, and 630 nm against a 90% acetone blank. Following this, the samples were acidified and remeasured at 750 and 663 nm. A Hitachi Perkin-Elmer Model 139 UV-VIS Spectrophotometer with a photomultiplier unit was utilized at less than a 1 nm bandwidth. Biomass determinations were calculated from the other half of the split samples utilizing dry weight and organic weight (ash-free dry weight) measurements (Strickland and Parsons, 1972c; Soeder *et al.*, 1974). Dry weight determinations were made after desiccation on standard GF/C glass fiber filters over a drying agent. Thereafter the samples were ashed at 475°C to complete the organic weight determinations.

The ratio of organic matter to chlorophyll α concentration has been used as an indicator for detecting changes in community structure. This index, as modified by Weber (1973), is calculated as follows:

$$\text{Autotrophic Index} = \frac{\text{Organic weight (mg/m}^2\text{)}}{\text{Chlorophyll } \alpha \text{ (mg/m}^2\text{)}} .$$

Our ecosystems were characterized by this index for comparative evaluation.

For evaluation of the effects of enhanced UV-B radiation on community composition, diatoms were allowed to develop on acrylic plates at depths of 18 and 34 cm in both chambers for 2, 4, and 6 weeks during the Winter study, 3 and 6 weeks during the Spring study and 1, 2, and 4 weeks during the Summer study. Samples were scraped off and slides prepared by the method described by Hendey (1974). Approximately 500 diatoms in each sample were evaluated taxonomically. Two indices of diversity were utilized for an analysis of community composition: an estimator of the Shannon-Wiener Information measure (H'') expressed as nats per individual (McIntosh, 1967)

and a measure of Redundancy (R'). The estimator for the Information measure was derived from

$$H'' = - \sum_{i=1}^S (n_i/N) \log_e (n_i/N) ,$$

where n_i is the number of individuals in the i -th taxon, N is the number of individuals in the sample, and S is the number of taxa represented in the sample. H'' ranges from 0 ($\log_e 1$), if all of the individuals in the assemblage are of one taxon, to $\log_e N$, if the number of taxa equals the number of individuals. The more taxa there are and the more nearly equal their proportions, the greater the uncertainty of predicting the taxon of the next individual to be observed and, therefore, the greater the diversity. Pielou (1966a, 1966b), McIntosh (1967) and Lloyd *et al.* (1968) have discussed the advantages, disadvantages, uses and misuses of the Information index of diversity. The cautions expressed by these authors have been taken into consideration in the present experiment.

To calculate a Redundancy index for a specific assemblage, a maximum and minimum diversity for the assemblage must be determined (Patten, 1962). As explained by McIntire and Overton (1971), a conditional maximum and minimum diversity based on the observed number of species in a sample can be derived from

$$H''_{\text{CMAX}} = \log_e S ,$$

and

$$H''_{\text{CMIN}} = - \left[\left(\frac{S-1}{N} \right) \log_e \left(\frac{1}{N} \right) + \left(\frac{N-S+1}{N} \right) \log_e \left(\frac{N-S+1}{N} \right) \right] .$$

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The results of these calculations were used to determine the Redundancy index, where

$$R' = \frac{H''_{\text{CMAX}} - H''}{H''_{\text{CMAX}} - H''_{\text{CMIN}}} .$$

The Redundancy index expresses the degree of dominance in a given sample relative to the partitioning of individuals among the taxa. Values of R' range from 0, when the individuals are equally distributed among the taxa, to 1, when all but one taxon are represented by a single individual.

A comparison of assemblages obtained from each of the two chambers was determined by utilizing the Difference measure of MacArthur (1965),

$$D_{hk} = \exp (H''_T - \bar{H}'') ,$$

where H''_T is the Information measure for the combined h -th (surface exposure of 1.0 W/m^2) and k -th (surface exposure of 1.4 W/m^2) assemblages treated as one assemblage by a weighted summation, and \bar{H}'' is the mean diversity for the two individual assemblages. These are determined from

$$H''_T = - \sum_{i=1}^S \frac{(n_{ih}/N_h + n_{jk}/N_k)}{2} \log_2 \frac{(n_{ih}/N_h + n_{jk}/N_k)}{2} ,$$

and

$$\bar{H}'' = \frac{(H''_h + H''_k)}{2} .$$

If the taxa composition and relative abundances are the same, D_{hk} has the minimum value of 1, and if the assemblages possess no taxa in common, D_{hk} has the maximum value of 2.

RESULTS

Winter 1976-1977

With time, a significant difference in weekly chlorophyll α concentrations developed between the two chambers (chlorophyll determinations of aliquots of the same sample averaged within six percent of the mean). As the experiment progressed, the chamber exposed to the lower level of UV-B radiation developed significantly higher chlorophyll α concentrations than the other chamber (Fig. 3). An F-test of the adjusted means of chlorophyll α concentrations (versus organic weight) for the 42 samples (six samples per week; seven weeks) taken at a depth of 41 cm resulted in $F_{1,39} = 9.089$ ($p < 0.01$), and for the samples taken at a depth of 26 cm, $F_{1,39} = 24.859$ ($p < 0.001$) (Snedecor and Cochran, 1967). As can be determined from Figures 3 and 4, there is a progressive shift during the series of seven sampling periods to a marked increase in biomass (organic weight) of benthic forms in the chamber exposed to the lower level of UV-B radiation. The Autotrophic Indices reflect these changes in biomass and chlorophyll α concentrations (Fig. 4).

Microscopic study revealed diatom species to be, by far, the dominant autotrophic component of the samples analyzed. Comparative analyses of species diversity within the two chambers indicated that higher levels of UV-B radiation resulted in a less diverse community structure (Tables 1 and 2). This is illustrated by the Information index, the measure of Redundancy, and the list of dominant, attached diatoms. Also, the Difference measure increased as the experiment progressed.

Apparent absorption coefficients of the seawater (Table 3) were derived from readings taken with the submersible R-B Meter at depths of 0, 33, 41, and 49 cm. There was an increase of the coefficients for the

chamber with a surface exposure of 1.0 W/m^2 relative to that for the chamber receiving 1.4 W/m^2 .

DISCUSSION

At the onset of our experiment the flow-through chambers simulate denuded, sloping estuarine environments. Potentially sessile organisms are recruited from open water and, with time, recruitment also occurs from nearby occupied areas. This type of recruitment is also found in nature. Differences in biomass and chlorophyll a concentrations between the two chambers thus reflect the cumulative effects of UV-B enhancement on the experimental ecosystem as a whole, as well as the UV-B effects on those newly recruited organisms settling on the sampling plates during any given week. The insignificant differences in chlorophyll a during the first four weeks indicate no significant depression of photosynthetic organisms by enhanced UV-B radiation. Only after five weeks does one see a significant depression of chlorophyll production or photosynthetic organisms. As indicated by the Autotrophic Indices, this reduction in chlorophyll could result from a progressive community structure shift to non-photosynthetic organisms. By the fifth week the difference in Autotrophic Indices between the two chambers is extremely significant and appears to have stabilized. The community structure shift to non-photosynthetic forms could induce a detrimental effect on an ecosystem.

Although no diatom diversity studies were carried out in Yaquina Estuary during this series of sample collections from the experimental chambers, McIntire and Overton (1971) have compiled some representative data from the estuary for 1968 and 1969. The mean number of species identified for a sample size of approximately 500 was 39, their mean Information measure (H'') was 2.234 nats/individual, and the mean Redundancy measure (R') was 0.309. These figures are not significantly different from

those obtained from the chamber receiving the lower level of UV-B radiation.

As has been demonstrated for certain species, present levels of UV-B radiation have deleterious effects upon organisms (Committee on Impacts of Stratospheric Change, 1976), and many exhibit differential sensitivity to these levels. The effect of enhanced levels of UV-B radiation, or any other environmental stress, upon the structure of a community may be irreversible, which is a consequence of more significance than the effects upon total biomass or concentration of photosynthetic pigments. In nature a shift in structure similar to that found in our experimental chambers could express itself by changes in the kinds and numbers of consumer organisms resulting from disturbances of food chain relationships and concurrent disruptions of competitive equilibria and predator-prey interactions.

Wilhm and Dorris (1968) have reported several occurrences where chemical stress factors have resulted in a reduction of the species diversity of benthic macroinvertebrates and other benthic fauna. Also, Bechtel and Copeland (1970) have used the depression of species diversity as an indicator of environmental stress in the Galveston Bay area of Texas. If irreversible shifts were to occur in the types of organisms found at the base of a marine food web, dominant forms might arise which could cause drastic changes in the structure of the entire ecosystem. The dominant form which develops in an ecosystem may be repressive and, under limiting conditions or conditions of stress, lead to a deterioration in diversity (Hulburt, 1970). Energy transfer through an ecosystem could be significantly altered if the forms selected for by additional UV-B stress

were of a different size, were less digestible, or were nutritionally deficient in some micronutrient (Provasoli *et al.*, 1959). This could result in a deficient energy supply for organisms at higher trophic levels, especially for the economically important fish and shellfish near the top of a food pyramid. Thus, the fate of estuarine animals is strongly linked to the other community constituents that also inhabit the biotope, and any major modification in the species composition and/or productivity of autotrophs is a potential threat to the stability and perpetuation of the entire ecological unit.

To have a direct effect upon marine organisms, UV-B radiation must penetrate a medium of widely varying transmission. The penetration depends greatly upon the concentration of dissolved organic matter in the seawater as well as the amount of suspended material in the water column. Jerlov (1968) has classified various optical water types occurring in nature and has tabulated their pertinent transmittance characteristics. Diffuse attenuation coefficients, k , for the wavelength of maximum transmission for the various water types can be used to calculate the depth of the euphotic zone (the depth at which photosynthetically active radiation is reduced to one percent of its surface value).

The smallest or most conservative estimate of potential damage due to increased levels of UV-B radiation within the euphotic zone will occur in that region which would receive radiation levels greater than that presently received at the surface. Zaneveld (1975) has called this region the "minimum zone of impact." The depth of this zone is calculated from:

$$z_1 = \frac{\log_e (E_1)}{k_{UVB}},$$

where z_1 is the depth of the minimum zone of impact, E_i is the relative irradiance of the enhanced surface UV-B radiation, and k_{UVB} is the diffuse attenuation coefficient of the water as measured within the UV-B region. From the depth of the minimum zone of impact and the distribution of plankton in the water column the percentage of productive phytoplankton which would be affected by increased UV-B irradiance can be determined. Zaneveld calculated that a 25% decrease in stratospheric ozone would cause 3 to 10 percent of the productive phytoplankton in the oceans to receive larger amounts of UV-B radiation than are now being received at the surface. In his calculations he assumes that a 25% decrease in stratospheric ozone concentration will result in a 28% increase in the amount of 310 nm radiation reaching the surface of the earth. The choice of 310 nm radiation was based on the most extensive data available at that time for transmission of UV-B radiation through natural waters (Jerlov, 1968). The 28% increase is based on the calculations of Shettle and Green (1974) for 310 nm downward global flux at zero zenith angle, although the percent increase rises markedly with increasing zenith angle.

The use of one wavelength, as by Zaneveld, obscures the fact that a 25% ozone reduction yields a substantially greater increase in total biologically effective UV-B radiation because of the greater effectiveness of wavelengths below 310 nm. A more realistic conversion would be about a 50% increase in total biologically effective UV-B radiation at the surface for a 25% decrease in ozone (Panel on Atmospheric Chemistry, 1976). On the other hand, the seawater attenuation coefficients for the shorter wavelengths in the UV-B region are greater than that for 310 nm (Zaneveld, 1975) so the increased biologically effective UV-B radiation under water would be

less than 50%. The available seawater absorption coefficients for the various UV-B wavelengths vary widely and/or are not sufficiently adequate to allow the calculation of the actual increase in biologically effective UV-B radiation.

In order to predict the potential impact of enhanced UV-B irradiance, the relative effectiveness of that radiation should be determined. The critical targets should show a response in the 290-320 nm waveband, the region affected by an ozone decrease. There is essentially no radiation shorter than 290 nm that reaches the surface of the earth to affect molecules which absorb at shorter wavelengths. For molecules which absorb at wavelengths longer than 320 nm, the intensity of radiation in that region would not be greatly affected by an ozone decrease. Green and Miller (1975) have calculated an analytic representation of two biological sensitivity functions: (1) the long-wavelength tail of a DNA action spectrum compiled by Setlow (1974),

$$\epsilon_{\text{DNA}}(\lambda) = \exp \left\{ k \left[\frac{1}{1 + \exp [(\lambda - \lambda_o)/\lambda_f]} - 1 \right] \right\},$$

where $k = 13.82$, $\lambda_o = 310$, and $\lambda_f = 9$, and (2) Caldwell's (1968) generalized action spectrum,

$$\epsilon_c(\lambda) = A \left[1 - (\lambda/\lambda_c)^n \right] \exp - \left[(\lambda - \lambda_o)/\lambda_f \right],$$

where $A = 2.618$, $n = 2$, $\lambda_c = 313.3$, $\lambda_o = 300$, and $\lambda_f = 31.08$. Caldwell compiled data on inhibition of photosynthesis, mutation in spores and a fungus, frequency of endosperm deficiencies, a germicidal action spectrum, epidermal cell damage, and induction of chromosomal aberrations. Weighting the irradiance at a particular wavelength by the relative effectiveness of

that wavelength, over all wavelengths from 290 nm to 320 nm, yielded the effective surface irradiances of our exposure regimen. For the chamber receiving a surface UV-B irradiance of 1.0 W/m^2 the total biologically effective surface irradiances were $1.36 \times 10^{-2} \text{ Eff W/m}^2$ (Setlow) and $9.65 \times 10^{-2} \text{ Eff W/m}^2$ (Caldwell). For the other chamber it was $1.84 \times 10^{-2} \text{ Eff W/m}^2$ (Setlow) (a 35% increase) and $1.35 \times 10^{-1} \text{ Eff W/m}^2$ (Caldwell) (a 40% increase).

Based on the apparent absorption coefficients of the water column in the chamber exposed to enhanced UV-B radiation ($2.352 - 1.620 \text{ m}^{-1}$) and the relative enhancement of biologically effective surface irradiance ($1.35 - 1.40$), the minimum zone of impact would extend to at most 20.8 cm because the R-B Meter tends to overestimate the penetration of the short wavelength components of biologically effective UV-B radiation (Smith and Calkins, 1976). All of the samples from the two chambers were taken at depths equal to, or greater than, 18 cm. The significant differences which occur between the two chambers at depths greater than 20.8 cm point out that enhanced levels of UV-B radiation at the surface will result in increases throughout the water column and that these increased levels quite likely have effects on the organisms located at depths greater than the minimum zone of impact.

Thus, in order to avoid increased exposure to UV-B radiation, organisms throughout the entire water column would be required either to migrate downward a distance equal to the minimum zone of impact or to develop a UV screen. There are innumerable problems associated with a downward shift in position within the water column. Among these would be an alteration of both competitive and symbiotic interaction among organisms,

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a reduction of photosynthetically active radiation, and an overall resultant niche modification. In all cases discussed, there is a potential for an adverse effect of increased UV-B radiation..

RESULTS

Spring 1977, Summer 1977

No significant effects of UV-B radiation were noted for both the spring and summer studies involving the indoor flow-through chambers. These studies were carried out at reduced UV-B irradiance levels. Both the pigment and biomass analyses fluctuated sufficiently to produce inconclusive results. Also, for the spring study the differences in community diversity between the two chambers were insignificant as opposed to the significant differences which developed at the higher irradiance levels in the Winter 1976-1977 study (Tables 1, 2, 4, 5). The species enumeration has not been completed for the summer study.

DISCUSSION

Utilizing the spectroradiometric readings from 1976 it was determined that, although the absolute surface irradiance in the UV-B region for the experimental chambers was comparable to natural levels, the biological effectiveness of the radiation must be greater due to the relatively high intensity of the experimental irradiation at wavelengths around 290 nm. Based on two biological sensitivity functions (Green and Miller, 1975; Setlow, 1974; Caldwell, 1968) the surface irradiance of the experimental chambers for the spring and summer studies was reduced to simulate a natural *biologically effective* global exposure. The resultant lack of significant biological differences between the two experimental chambers has been noted.

There are two likely explanations for these results. First, there is a differential attenuation by the seawater of the radiation within the UV-B waveband. The wavelengths that are weighted most heavily in the two biological sensitivity functions are the wavelengths that are attenuated most strongly by the seawater. For solar radiation, the biologically effective irradiance spectrum peaks at approximately 308 nm (Caldwell function); whereas the peak is 302 nm for the experimental chambers (Fig. 5). A comparable difference occurs with the Setlow sensitivity function. Therefore, the farther down in the water column one measures, the greater the reduction of the biologically effective irradiance within the experimental chambers as compared with levels which would occur in a chamber exposed to *solar* UV-B radiation.

If one assumes that populations of organisms are capable of withstanding current, natural levels of UV-B radiation at the depths of interest, then the relatively low, effective UV-B irradiance of our experimental

design might not cause any detectable differences between the two chambers.

There are two possible actions being considered by our laboratory to resolve this alternative:

1. Procure a better solar simulator -- one which more closely approximates the solar spectrum in the UV-B region, and
2. Determine the absolute irradiance at the depth of interest and adjust the experimental surface irradiance to give the desired biologically effective exposure at that depth. Time and economic considerations favor this latter procedure.

The second likely explanation for the difference in results between the Winter 1976-1977 study and the spring and summer studies is based on a probable differential UV sensitivity of the common species found during the different seasons. Those organisms which are found in abundance during the winter months might be more UV sensitive than those found at other times of the year due to the decreased UV stress on the reproduction and development of those species. Sensitivity studies with the appropriate species should resolve this as an alternative possibility.

AREA 2

Work accomplished to date in our laboratory indicates that simulated solar UV-B radiation at present day surface irradiances as well as enhanced irradiances can kill marine algae, reduce photosynthesis, reduce growth in biomass, and can contribute to potentially adverse alterations in community structure. Diatoms are the primary autotrophic constituents of these microcosms, and survival studies utilizing representative unialgal cultures from our systems indicate that these organisms have the capacity to photoreactivate some of the potentially lethal, ultraviolet damage.

The intensity of photoreactivating radiation found in nature is an order of magnitude greater than that available in our previous laboratory conditions. Therefore, the objective of the following research was to obtain, *under natural light conditions*, data which will permit assessment of the potential impact upon key community components of the ecosystem due to increased UV-B radiation, and relating this impact to ecosystem perturbation.

MATERIALS AND METHODS

A fiberglass greenhouse containing three 800-liter flow-through tanks was constructed on the property of the O. S. U. Marine Science Center, Newport, Oregon. Fiberglass acts as a cut-off filter, transmitting natural, photoreactivating radiation longer than 380 nm. Seawater, drawn from lower Yaquina Estuary, flowed through a head tank which served as the source of seawater for the experimental tanks. This water provided for the natural recruitment of organisms into the tanks. The flow rate through the experimental tanks was approximately 0.5 l/min.

Sunlamps (Westinghouse FS40) were used to supply supplementary UV radiation for the tanks. The sunlamps for one of the tanks had aluminum reflectors and were covered by a 0.25 mm thickness of Mylar (a 315 nm cut-off filter). The sunlamps for a second tank also had aluminum reflectors, but were covered by a 0.25 mm thickness of cellulose acetate (a 290 nm cut-off filter). The sunlamps for the third tank were also covered with cellulose acetate, but there were no reflectors behind the lamps. This resulted in one tank receiving almost no UV-B radiation (0.01 W/m^2); another was exposed to a surface UV-B irradiance of 0.77 W/m^2 , and the third to an irradiance of 0.56 W/m^2 . The sunlamps were "on" for nine hours daily (0800-1700 h) (Table 6).

Enumeration of viable "total" heterotrophic microorganisms from each of the experimental tanks, as well as the proportion of chromagens was determined by the traditional plate count technique. Serial dilutions (10^0 , 10^{-1} , 10^{-2}) of samples collected at a depth of 15 cm were prepared in sterile seawater, spread-plate on Bacto-Marine Agar 2216 (Code 0979, Difco Laboratories, Detroit, Michigan) and incubated in the dark for seven days. One-half of the plates were incubated at 12°C and the others at 25°C .

Colony counts were made on the second and seventh day.

Also, seawater samples (1500-2000 ml) from tank depths of 15 cm and 74 cm were filtered through 4.25 cm GF/C glass-fiber filters and analyzed by the SCOR/UNESCO technique for concentration of chlorophyll *a*. A Coleman 124D Double Beam Spectrophotometer with a 10 cm cell-path-length was utilized.

DISCUSSION

Although the planktonic biomass in the estuary was low at the time of the experiment (October-November), the depression of growth of planktonic algae by UV-B exposure was noticeable. As has been demonstrated previously, the planktonic forms are sensitive to the stress of UV-B radiation, even at current natural levels. The calculated integrated daily solar exposure in the 290-315 nm waveband at 45°N in October is 15.5 kJ/m² (Johnson *et al.*, 1976). It is interesting to note that the chlorophyll *a* concentration in the seawater inflow (1.85 mg/m³), which has been exposed to approximately 15.5 kJ/m²/d of UV-B radiation, was identical to the chlorophyll *a* concentration in the tank exposed to an equivalent daily UV-B exposure (18.1 kJ/m²). This could lead to the speculation that, if all other factors were the same, a 38% increase (24.9 kJ·m⁻²/18.1 kJ·m⁻²) of UV-B exposure in an estuarine ecosystem would result in a 4% decrease (1.77 mg·m⁻³/1.85 mg·m⁻³) in primary productivity (Table 6). Obviously, more work needs to be done to confirm these results.

The increase in the relative incidence of chromagenic bacteria in the flow-through tanks exposed to UV-B radiation is of equal significance. Previously, a similar increase in the proportion of chromagenic bacteria resulting from UV-B stress had been reported in batch cultures of estuarine water (Van Dyke and Thomson, 1975). Many pigmented forms are pathogenic to economically and/or ecologically important marine organisms (e.g., oysters) (Guillard, 1959; Tubiash *et al.*, 1965). Also, an alteration of the composition of the bacterial community could affect the recycling of nutrients, which would have an immediate effect on primary productivity.

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AREA 3

Sensitivity studies were concentrated on the marine primary producer, *Melosira nummuloides*, and the marine copepod, *Acartia clausi*. *M. nummuloides* is a centric, chain-forming diatom that can be found both in the water column and attached to surfaces. McIntire and Wulff (1972) found this diatom to be among the thirty-five most abundant species of attached diatoms associated with Yaquina Estuary. *A. clausi* is important in coastal and estuarine zooplankton communities throughout the Northern Hemisphere. It is found in such places as the Mediterranean Sea, the Black Sea, the Atlantic coast of Europe, both coasts of North America, and in the waters around Japan.

MELOSIRA NUMMULOIDES

MATERIALS AND METHODS

A stock population of *M. nummuloides* was maintained in our laboratory in liquid culture (f/2 enriched seawater, Guillard and Ryther, 1962) at 13°C. To keep the culture in an active growth phase the stock was sub-cultured every ten to fourteen days. Visible radiation was supplied to the stock culture by "deluxe-white" fluorescent lamps (Vita-Lite, Duro-Test Corp., North Bergen, NJ) for nine hours daily. Supplemental UV radiation was supplied by fluorescent sunlamps (Westinghouse FS40) during a five-hour cycle centered in the visible photoperiod. A 0.13 mm thickness of presolarized cellulose triacetate or cellulose acetate film covering the lamps served as a 290 nm cut-off filter. The filter was changed routinely to compensate for measured photodegradation. The daily exposure in the

290-315 nm waveband at the surface of the stock culture was 2.2 kJ/m^2 . The instrumentation for determining the spectral irradiance has been described in detail elsewhere (Worrest *et al.*, 1978). Briefly, the instrument is a modified Gamma Scientific 2900SR Spectroradiometer System which has been characterized by the U. S. National Bureau of Standards.

To obtain chains of *M. nummuloides* for experimental irradiation the stock culture was mixed well and a portion withdrawn and diluted in an Erlenmeyer flask containing sterile f/2 enriched seawater. The flask was swirled vigorously by hand to break up the longer chains. Aliquots (approximately 0.05 ml) of this mixture were spot-plated onto f/2 enriched seawater agar plates. The square plates (10 x 10 cm) were divided into two regions with one row of three spots being placed in the center of each region. All chains greater than 20 cells in length were removed from each of the spots, leaving one or two chains per spot. The result was six spots per plate containing a total of six to twelve chains each having 4-20 cells. Ten plates were prepared for each experiment. The number of cells in each chain was determined and then the plates were covered and incubated overnight. As a check for growth the chains were counted again the next morning prior to being irradiated. Those chains which gave no evidence of growth overnight were not considered in the experimental results. Greater than 95% of all chains survived the transfer technique.

The covers of the petri dishes were cut and modified to accommodate two pieces of filter material (9.5 x 5 cm) so that the two regions on each plate could receive different conditions of irradiation simultaneously. Three types of filters were used to achieve the various levels of

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irradiation. One type was a 0.13 mm thickness of cellulose triacetate film (Kodacel) which had been presolarized for 36 hours. The irradiance through this filter was 0.93 W/m^2 in the 290-315 nm waveband. Further photodegradation for an additional twelve hours diminished the irradiance to 0.88 W/m^2 . Another filter was a 0.19 mm thickness of 36-hour Kodacel and this gave an irradiance of 0.66 W/m^2 . This degraded to 0.64 W/m^2 at 48 hours. The third filter was a 0.13 mm thickness of Mylar 'S' which gave an irradiance of 0.01 W/m^2 in the 290-315 nm waveband.

The plates were exposed to "deluxe-white" fluorescent lamps for nine hours daily. UV-B exposure, supplied by FS40 fluorescent sunlamps, was centered in the visible photocycle. While eight regions of the plates were being irradiated through 0.19 mm Kodacel another eight regions were receiving a 41% enhancement (0.13 mm Kodacel). There were also four regions being irradiated through the Mylar filters which served as controls for UV-B effects. The plates were irradiated on a turntable for three days. The chains of *Melosira* were then counted and a growth rate (k) was calculated:

$$k = \ln \frac{N_1}{N_0} \left(\frac{1}{t \ln 2} \right),$$

where N_1 is the number of cells in a filament at the end of three days, N_0 is the number of cells at the beginning of irradiation, and t is the duration of the experiment in days. Chains shorter than 200 cells were counted and a growth rate was determined (k_1). Chains shorter than 80 cells were counted, the growth rate determined (K_1), and then they were

irradiated for a second three days. A second three-day growth rate (k_2) was calculated for these chains.

Three different experiments were carried out, each with a different length of UV-B exposure. The exposure periods were 1.5, 2.5, and 4 hours. The plates exposed to supplemental UV-B radiation for 1.5 hours daily were irradiated for three days, k_1 was determined, and then the plates were exposed for a second three days to determine k_2 . In the next experiment the plates were exposed for 2.5 hours daily and k_1 was determined. Fresh 36-hour presolarized Kodacel was placed in the plate covers, turned 180° and irradiated for a second three-day period. The purpose of rotating the plate covers 180° was to change the dose rate by placing a different filter over each region on the plates (e.g., a 0.13 mm thickness of Kodacel covered a chain of cells where a 0.19 mm thickness had been located previously). The chains were counted again and k_2 was determined. In the third study the plates were irradiated for 4 hours daily for three days and k_1 was calculated. Fresh 36-hour presolarized filters were placed in the covers and some were turned 180°, but some were left in the original position. Following three additional days of exposure, k_2 was determined.

RESULTS

Cells in treatment group A (Table 7) in the three different experiments (sunlamps "on" for 1.5, 2.5 and 4 hours daily) were covered by Mylar filters and the growth rates for these chains were consistently high (approximately 0.93 divisions/day). Exposure to increased levels of UV-B radiation caused significant reductions in both k_1 and k_2 . Those chains

that were exposed to a fluence rate of 0.66 W/m^2 for the first three days (k_1) and then to 0.93 W/m^2 during the second three days (k_2) showed a significant decrease during the second three days. When the reverse procedure was followed (0.93 W/m^2 followed by 0.66 W/m^2), the growth rate increased significantly during the second three days (k_2). In general, as the length of daily exposure at each fluence rate increased, the corresponding growth rates decreased.

DISCUSSION

These results confirm work that we have reported earlier: that daily exposure to UV-B radiation inhibits the growth of the marine diatom, *Melosira nummuloides*. Also it appears as if this organism has the capacity to recover from the inhibitory effects of a short-term exposure to enhanced levels of UV-B radiation. Within the six-day period, in no case did it appear as if the diatom was able to recover from the initial, enhanced stress of UV-B radiation while the enhanced stress continued.

ACARTIA CLAUSI

MATERIALS AND METHODS

Acartia clausi was isolated from Yaquina Estuary, Newport, Oregon. Stock cultures were reared in three-liter containers at approximately 15°C with a concentration of adults at approximately 500-600 individuals/container. The cultures were constantly aerated and fed a mixture of *Platymonas suecica*, *Thalassiosira pseudonana*, and *Isochrysis galbana*. To obtain larvae for irradiation studies, adults were placed in a liter beaker containing sterile

seawater and food. To collect eggs for experimentation the cultures were left for 12 hours at approximately 19°C. The eggs were gathered and placed in the dark for 42 hours. *A. clausi* eggs require light to hatch; therefore, at the end of 42 hours of darkness nearly all of the eggs were in the same physiological state just prior to hatching (Landry, 1976). Following illumination, most of the eggs (75-80%) hatched within 12 hours. The development of the larvae was reasonably synchronized allowing individual stages of naupliar development to be studied by waiting an appropriate length of time. Sixteen hours post-illumination yielded a mixture of N1 and N2 nauplii. Four days resulted in N3 and N4 nauplii and six days yielded N5 and N6 nauplii. Older stages of copepodites and adults could be separated directly from stock cultures. A sample from the animals to be irradiated was fixed and staged for each survival study.

The animals were irradiated in 20 ml of sterile seawater contained in 5 x 1.5 cm glass petri dishes. At least three replicates, each containing approximately 30 animals, were used at each exposure level. The petri dishes were placed on a turntable and exposed to radiation from a combination of "deluxe-white" fluorescent lamps and FS40 sunlamps. Each dish was covered by either a 0.25 mm thickness of Mylar (a 315 nm cut-off filter) or a 0.25 mm thickness of Kodacel (a 290 nm cut-off filter). The Kodacel had been presolarized for 18 hours. The irradiance in the 290-320 nm waveband under the Mylar filter was 0.02 W/m² and under the Kodacel it was 1.45 W/m².

Following exposure each petri dish was emptied into a separate 600 ml beaker containing a mixture of sterile seawater (400 ml) and *Isochrysis* stock culture (35 ml). The beakers were aerated and the larvae cultured

until some of the survivors reached the adult stage. At this point the survivors were counted. Also, equal numbers of survivors from various larval exposure groups were cultured and any offspring produced were counted. Equal numbers of males and females were irradiated in the C4-Adult group.

RESULTS

Using two-way analysis of variance it was determined that the percent survival between different exposure groups was significantly different ($p < 0.05$) (Figs. 6-9). There was an inverse relationship between total exposure and percent survival. In general, the older the animals were the more resistant they were to UV-B radiation. The major exception to this are the N5-N6 nauplii. This may be due to the fact that at this stage the animals are starting to go through a major metamorphosis into the first copepodite stage and, therefore, they may be more sensitive to environmental stress.

The number of offspring produced by the irradiated organisms was reduced. The relation can be described by a linear regression relating the number of offspring produced per survivor at each exposure level versus the total exposure (Fig. 10). Transformations of the linear regression were tried, but the correlation coefficients were lower.

In the study where equal numbers of males and females were irradiated (C4-Adult) regression analysis showed that the males were more sensitive to UV-B irradiation than were the females ($p < 0.05$); whereas the survival of the control animals (0.0 kJ/m^2) were not different ($p > 0.05$) (Fig. 11).

DISCUSSION

Among the exposure groups there were marked differences (1) in the number of organisms surviving two weeks into adulthood, (2) in the number of second generation organisms produced, and (3) in the number of offspring produced per surviving adult. This suggests that even when a copepod larva survives exposure to UV-B radiation its ability to produce offspring is reduced. This decrease combined with the decreased survival of the exposed organisms indicates that there is a potential for an adverse effect on the marine ecosystem resulting from increased UV-B radiation.

SUMMARY

Based on the results of the three areas of study it appears as if current natural levels of UV-B radiation, as well as enhanced levels, inhibit the development of some species of marine primary producers and consumers and can contribute to potentially adverse alterations in community structure. Threshold values of *potential* damage have apparently been exceeded in nature and any increase would be detectable on a species level were it not for the photoreactivation of the potential damage. The degree to which photoreactivation can alleviate the potential damage under natural conditions is the area of research which merits intense study. Our laboratory is now beginning to acquire data regarding the impact of UV-B radiation upon marine ecosystems under natural levels of photoreactivating radiation. These ecosystems will then be sampled over a period of time and analyzed for various community attributes. Since the estuary being simulated is located

near 45°N latitude and is thus subjected to seasonal fluctuations of physical conditions with associated changes in the estuarine biota, the emphasis will be on carrying out a series of studies to determine how the seasonally different estuarine communities respond to UV-B exposure. Under natural conditions there occur apparent differences in the seasonal species composition of the estuarine ecosystem. It is possible that the different communities exhibit different responses to UV-B stress. The refinement of instrumentation to obtain UV-B fluence rates under water would aid greatly in a more precise assessment of the impact of enhanced UV-B radiation upon these marine communities.

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Table 1. Analysis of community composition. In the chamber designated "Lo" the surface UV-B irradiance level was 1.0 W/m^2 , and in the chamber designated "Hi" it was 1.4 W/m^2 . Step 4 has a depth of 34 cm and step 6 has a depth of 18 cm. Diatom assemblages were allowed to develop 2, 4 and 6 weeks. N is the number of diatoms in the sample, S is the number of species represented in the sample, H'' is the estimator for the common Information index, R' is a measure of Redundancy, and D is a Difference measure.

<u>Chamber-Step-Week</u>	<u>N</u>	<u>S</u>	<u>H''(nats)</u>	<u>R'</u>	<u>D</u>
Lo-4-2	508	63	3.095	0.321	1.112
Hi-4-2	510	63	3.095	0.320	
Lo-4-4	499	45	2.840	0.304	1.228
Hi-4-4	510	31	1.942	0.496	
Lo-4-6	507	38	2.715	0.296	1.397
Hi-4-6	506	25	1.625	0.554	
Lo-6-2	507	59	2.893	0.364	1.134
Hi-6-2	507	69	3.218	0.310	
Lo-6-4	502	39	2.789	0.280	1.201
Hi-6-4	504	35	2.277	0.416	
Lo-6-6	504	39	2.640	0.328	1.367
Hi-6-6	504	25	1.514	0.593	

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Table 2. A list of the dominant diatom species ($\geq 5\%$ of the total number of enumerated, attached diatoms) and the percent composition of those species within each assemblage. In the chamber designated "Lo" the surface UV-B irradiance level was 1.0 W/m^2 , and in the chamber designated "Hi" it was 1.4 W/m^2 . Step 4 has a depth of 34 cm and step 6 has a depth of 18 cm. The diatom assemblages were allowed to develop 2, 4, and 6 weeks.

<u>Chamber-Step-Week</u>	<u>Species</u>	<u>Percent Composition</u>
Lo-4-2	<i>Melosira sulcata</i> (Ehr.) Kütz.	30%
	<i>M. nummuloides</i> (Dillw.) Ag.	14%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	9%
	<i>Navicula salinicola</i> Hust.	6%
Hi-4-2	<i>Melosira sulcata</i> (Ehr.) Kütz.	21%
	<i>M. nummuloides</i> (Dillw.) Ag.	16%
	<i>Amphora</i> sp. #2	13%
	<i>A. tenerrima</i> Al. & Hust.	5%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	5%
	<i>Stauroneis constricta</i> (W. Sm.) Cl.	5%
Lo-4-4	<i>Navicula salinicola</i> Hust.	28%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	17%
	<i>Nitzschia</i> sp. #5	7%
	<i>Amphora</i> sp. #2	6%
	<i>Fragilaria striatula</i> v. <i>californica</i> Grun.	6%
	<i>Stauroneis constricta</i> (W. Sm.) Cl.	5%
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i> (Rabh.) Grun.	5%
	<i>Nitzschia</i> sp. #63	5%
Hi-4-4	<i>Stauroneis constricta</i> (W. Sm.) Cl.	40%
	<i>Navicula salinicola</i> Hust.	23%
	<i>Amphora</i> sp. #2	11%
	<i>A. tenerrima</i> Al. & Hust.	10%
Lo-4-6	<i>Navicula salinicola</i> Hust.	21%
	<i>Fragilaria striatula</i> v. <i>californica</i> Grun. —	17%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	14%
	<i>Nitzschia</i> sp. #5	7%
	<i>N. frustulum</i> v. <i>perpusilla</i> (Rabh.) Grun.	6%
	<i>Amphora tenerrima</i> Al. & Hust.	6%
	<i>Melosira nummuloides</i> (Dillw.) Ag.	6%
Hi-4-6	<i>Stauroneis constricta</i> (W. Sm.) Cl.	58%
	<i>Amphora</i> sp. #2	15%
	<i>A. tenerrima</i>	5%

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<u>Chamber-Step-Week</u>	<u>Species</u>	<u>Percent Composition</u>
Lo-6-2	<i>Melosira sulcata</i> (Ehr.) Kütz.	36%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	12%
	<i>Cocconeis scutellum</i> Ehr.	7%
	<i>Melosira nummuloides</i> (Dillw.) Ag.	7%
Hi-6-2	<i>Melosira sulcata</i> (Ehr.) Kütz.	28%
	<i>Amphora</i> sp. #2	9%
	<i>M. nummuloides</i> (Dillw.) Ag.	8%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	7%
	<i>Stauroneis constricta</i> (W. Sm.) Cl.	6%
Lo-6-4	<i>Navicula salinicola</i> Hust.	24%
	<i>Fragilaria striatula</i> v. <i>californica</i> Grun.	14%
	<i>Amphora tenerrima</i> Al. & Hust.	10%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	7%
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i> (Rabh.) Grun.	7%
	<i>Stauroneis constricta</i> (W. Sm.) Cl.	7%
Hi-6-4	<i>Stauroneis constricta</i> (W. Sm.) Cl.	36%
	<i>Amphora</i> sp. #2	23%
	<i>Navicula salinicola</i> Hust.	9%
	<i>A. tenerrima</i> Al. & Hust.	7%
Lo-6-6	<i>Navicula salinicola</i> Hust.	24%
	<i>Fragilaria striatula</i> v. <i>californica</i> Grun.	19%
	<i>Synedra fasciculata</i> (Ag.) Kütz.	11%
	<i>Stauroneis constricta</i> (W. Sm.) Cl.	8%
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i> (Rabh.) Grun.	8%
	<i>Amphora tenerrima</i> Al. & Hust.	7%
	<i>A. sp. #2</i>	5%
Hi-6-6	<i>Stauroneis constricta</i> (W. Sm.) Cl.	69%
	<i>Amphora</i> sp. #2	10%

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Table 3. Apparent absorption coefficients in the UV-B region of the seawater in the two flow-through chambers as determined at four time intervals during the experiment. A submersible R-B Meter (CIAP model) was used to determine irradiance.

Time (Weeks)	Chamber	Apparent Absorption Coefficient (m^{-1})	Calculated Percent Surface Irradiance at 1 and 2 meters
2	Lo	1.962 ± 0.194	14 2
	Hi	1.802 ± 0.251	16 3
3	Lo	1.897 ± 0.288	15 2
	Hi	1.620 ± 0.220	20 4
5	Lo	3.372 ± 0.280	3 0.1
	Hi	2.352 ± 0.150	10 0.9
7	Lo	2.830 ± 0.490	6 0.3
	Hi	1.857 ± 0.410	16 2

Table 4. Analysis of community composition. In the chamber designated "Lo" the surface UV-B irradiance level was 0.42 W/m^2 , and in the chamber designated "Hi" it was 0.50 W/m^2 . Step 4 has a depth of 34 cm and step 6 has a depth of 18 cm. Diatom assemblages were allowed to develop 3 and 6 weeks. N is the number of diatoms in the sample, S is the number of species represented in the sample, H' is the estimator for the common Information index, R' is a measure of Redundancy, and D is a Difference measure.

<u>Chamber-Step-Week</u>	<u>N</u>	<u>S</u>	<u>H' (nats)</u>	<u>R'</u>	<u>D</u>
Lo-4-3	500	38	2.646	0.319	1.063
Hi-4-3	507	36	2.657	0.300	
Lo-4-6	513	39	2.763	0.288	1.037
Hi-4-6	506	33	2.507	0.325	
Lo-6-3	502	37	2.524	0.351	1.040
Hi-6-3	516	39	2.567	0.350	
Lo-6-6	508	32	2.658	0.267	1.038
Hi-6-6	513	40	2.658	0.328	

Table 5. A list of the dominant diatom species ($\geq 5\%$ of the total number of enumerated, attached diatoms) and the percent composition of those species within each assemblage. In the chamber designated "lo" the surface UV-B irradiance level was 0.42 W/m^2 , and in the chamber designated "hi" it was 0.50 W/m^2 . Step 4 has a depth of 34 cm and step 6 has a depth of 18 cm. The diatom assemblages were allowed to develop 3 and 6 weeks.

<u>Chamber-Step-Week</u>	<u>Species</u>	<u>Percent Composition</u>
Lo-4-3	<i>Nitzschia</i> sp. #3	30%
	<i>Navicula salinicola</i>	17%
	<i>N. directa</i>	11%
	<i>Nitzschia fontifuga</i>	10%
Hi-4-3	<i>Nitzschia</i> sp. #3	28%
	<i>Navicula directa</i>	15%
	<i>N. salinicola</i>	14%
	<i>N. sp. #2</i>	8%
	<i>Nitzschia fontifuga</i>	5%
Lo-4-6	<i>Navicula salinicola</i>	21%
	<i>Nitzschia</i> sp. #63	20%
	<i>Navicula</i> sp. #2	7%
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i>	6%
	<i>Navicula directa</i>	6%
	<i>Synedra fasciculata</i>	5%
	<i>Melosira nummuloides</i>	5%
	<i>Nitzschia fontifuga</i>	5%
Hi-4-6	<i>Navicula salinicola</i>	27%
	<i>Nitzschia</i> sp. #63	24%
	<i>Navicula</i> sp. #2	9%
	<i>N. directa</i>	5%
	<i>Synedra fasciculata</i>	5%
	<i>Fragilaria striatula</i> v. <i>californica</i>	5%

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<u>Chamber-Step-Week</u>	<u>Species</u>	<u>Percent Composition</u>
Lo-6-3	<i>Nitzschia</i> sp. #63	25%
	<i>Navicula salinicola</i>	24%
	<i>N. directa</i>	14%
	<i>Nitzschia fontifuga</i>	6%
	<i>Synedra fasciculata</i>	5%
Hi-6-3	<i>Navicula salinicola</i>	27%
	<i>Nitzschia</i> sp. #63	25%
	<i>Navicula directa</i>	9%
	<i>Nitzschia fontifuga</i>	8%
	<i>Navicula</i> sp. #2	6%
Lo-6-6	<i>Navicula salinicola</i>	25%
	<i>Nitzschia</i> sp. #63	19%
	<i>Navicula</i> sp. #2	7%
	<i>N. directa</i>	7%
	<i>Synedra fasciculata</i>	6%
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i>	6%
	<i>Amphora</i> sp. #40	5%
	<i>Melosira nummuloides</i>	5%
Hi-6-6	<i>Navicula salinicola</i>	25%
	<i>Nitzschia</i> sp. #63	20%
	<i>Navicula</i> sp. #2	9%
	<i>N. directa</i>	7%
	<i>Melosira nummuloides</i>	7%
	<i>Synedra fasciculata</i>	6%
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i>	5%

Table 6. Planktonic chlorophyll α concentration and enumeration of viable "total" heterotrophic microorganisms in flow-through ecosystem tanks exposed to three different levels of UV-B (290-315 nm) radiation.

DAILY EXPOSURE (290-315 nm)	CHLOROPHYLL α CONCENTRATION	BACTERIAL CONCENTRATION	PERCENTAGE BACTERIAL CHROMAGENS
0.3 kJ/m ²	2.44 mg/m ³	2.82 x 10 ³ /ml	6.3%
18.1 kJ/m ²	1.85 mg/m ³	1.13 x 10 ⁴ /ml	16.5%
24.9 kJ/m ²	1.77 mg/m ³	6.54 x 10 ³ /ml	37.2%
Seawater Inflow	1.85 mg/m ³	---	--

Table 7. Growth rates obtained for *Melosira nummuloides* during three experiments, each having a different length of daily UV-B stress (1.5, 2.5 and 4 h/day). Numbers in parentheses following growth rates represent numbers of chains counted.

TREAT- MENT GROUP	FLUENCE RATE (W/m ²)	GROWTH RATE (div./day)		
		1.5 h/day	2.5 h/day	4 h/day
A	0.01 (k ₁)	0.987 ± 0.094 (25)	0.894 ± 0.062 (23)	0.919 ± 0.073 (24)
B	0.66 (k ₁)	0.934 ± 0.077 (33)		0.642 ± 0.048 (11)
	0.66 (k ₂)	0.671 ± 0.062 (12)*		0.641 ± 0.041 (11)
C	0.93 (k ₁)	0.875 ± 0.083 (35)		0.394 ± 0.079 (12)
	0.93 (k ₂)	0.712 ± 0.049 (19)*		0.408 ± 0.057 (12)
D	0.66 (k ₁)		0.538 ± 0.088 (29)	0.649 ± 0.047 (35)
	0.93 (k ₂)		0.484 ± 0.072 (28)*	0.429 ± 0.045 (30)*
E	0.93 (k ₁)		0.512 ± 0.109 (29)	0.378 ± 0.075 (35)
	0.66 (k ₂)		0.590 ± 0.072 (25)*	0.633 ± 0.076 (33)*

*Significant difference between k₁ and k₂ (p < 0.001); all others,

p > 0.05.

Figure 1. Diagram of seminatural ecosystem chamber (continuous flow-through design) and associated water supply system.
Arrangement of radiation sources is illustrated.

CHAMBER DIMENSIONS

INSIDE WIDTH - 76 cm

INSIDE LENGTH - 3m

MAXIMUM INSIDE DEPTH - 65 cm

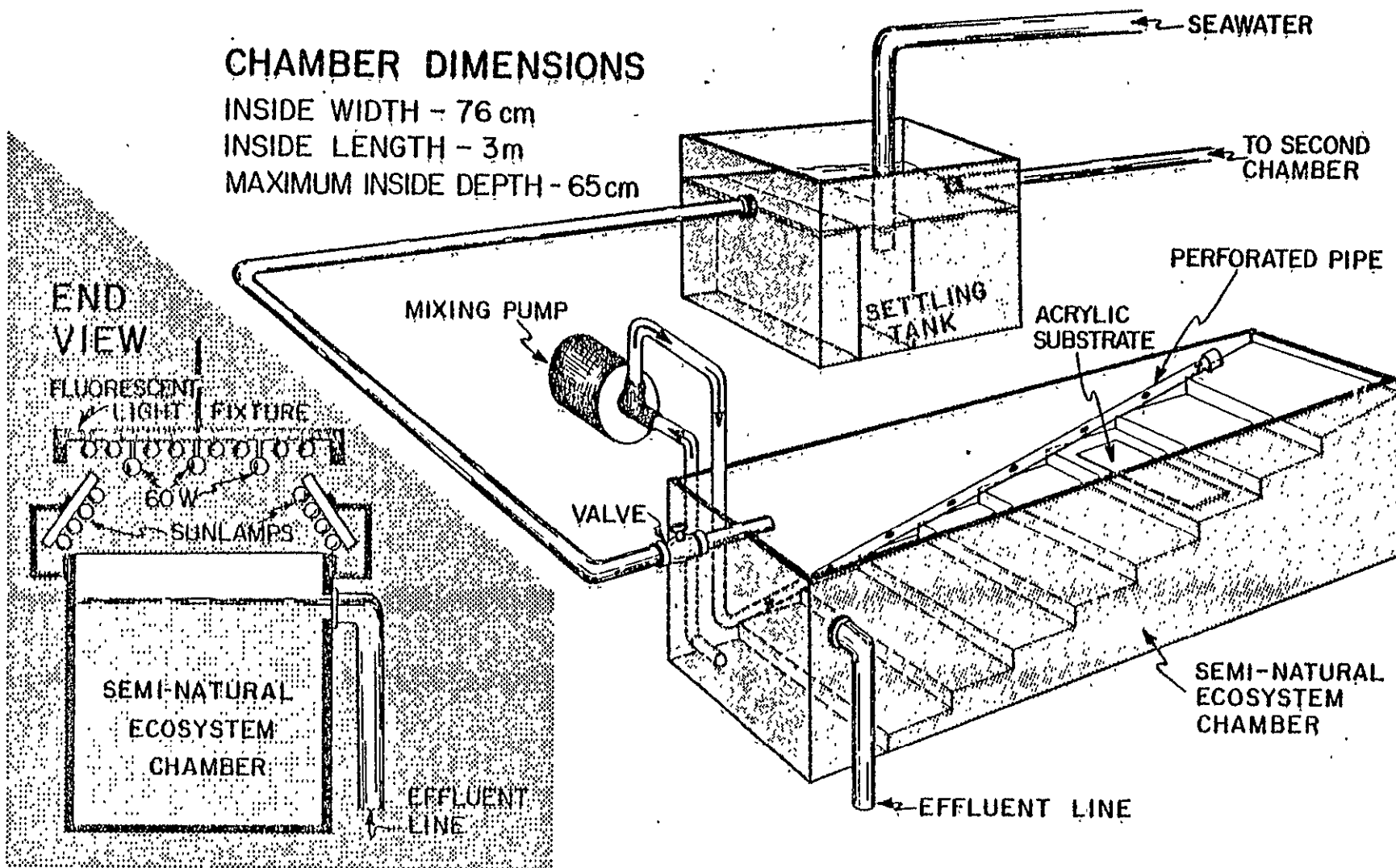


Figure 2. Irradiance spectra for the two chambers as measured at the water surface by a modified Gamma Scientific 2900SR Spectroradiometer system. Peak spectral irradiance in the 290-315 nm waveband for the chamber designated "Lo" was 1.0 W/m² and for the chamber designated "Hi," 1.4 W/m². The spectra for the two chambers at wavelengths greater than 370 nm were similar. Irradiance of characteristic mercury emission lines were not illustrated in the visible region.

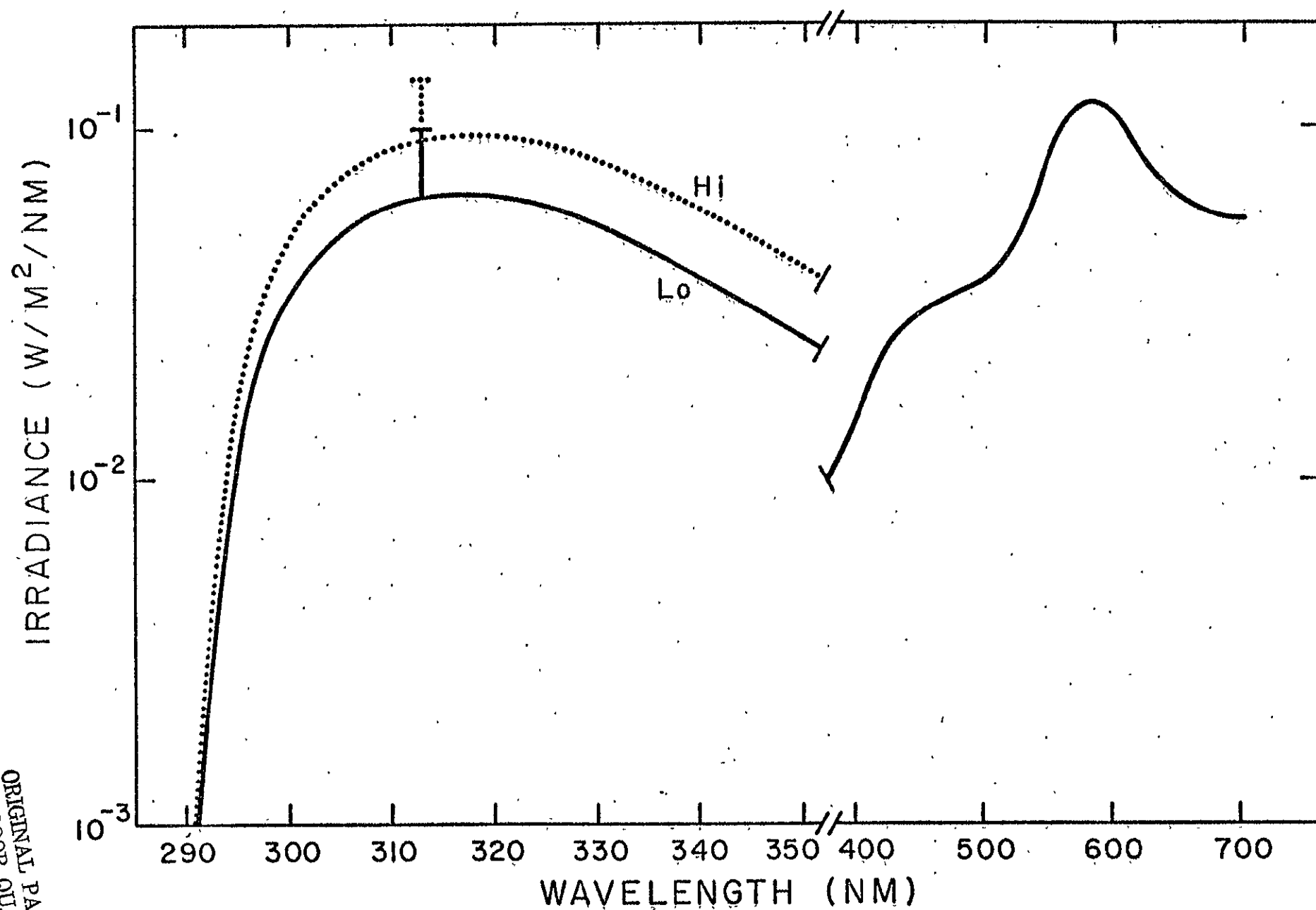


Figure 3. Chlorophyll a concentrations combined from samples taken at depths of 26 cm and 41 cm from the two flow-through chambers. Solid bars represent samples taken from the chamber receiving a surface irradiance of 1.0 W/m^2 (290–315 nm) and the shaded bars represent samples taken from the chamber receiving a surface irradiance of 1.4 W/m^2 . Error bars = ± 1 st. dev.; * = $p < 0.01$; all others, $p > 0.05$.

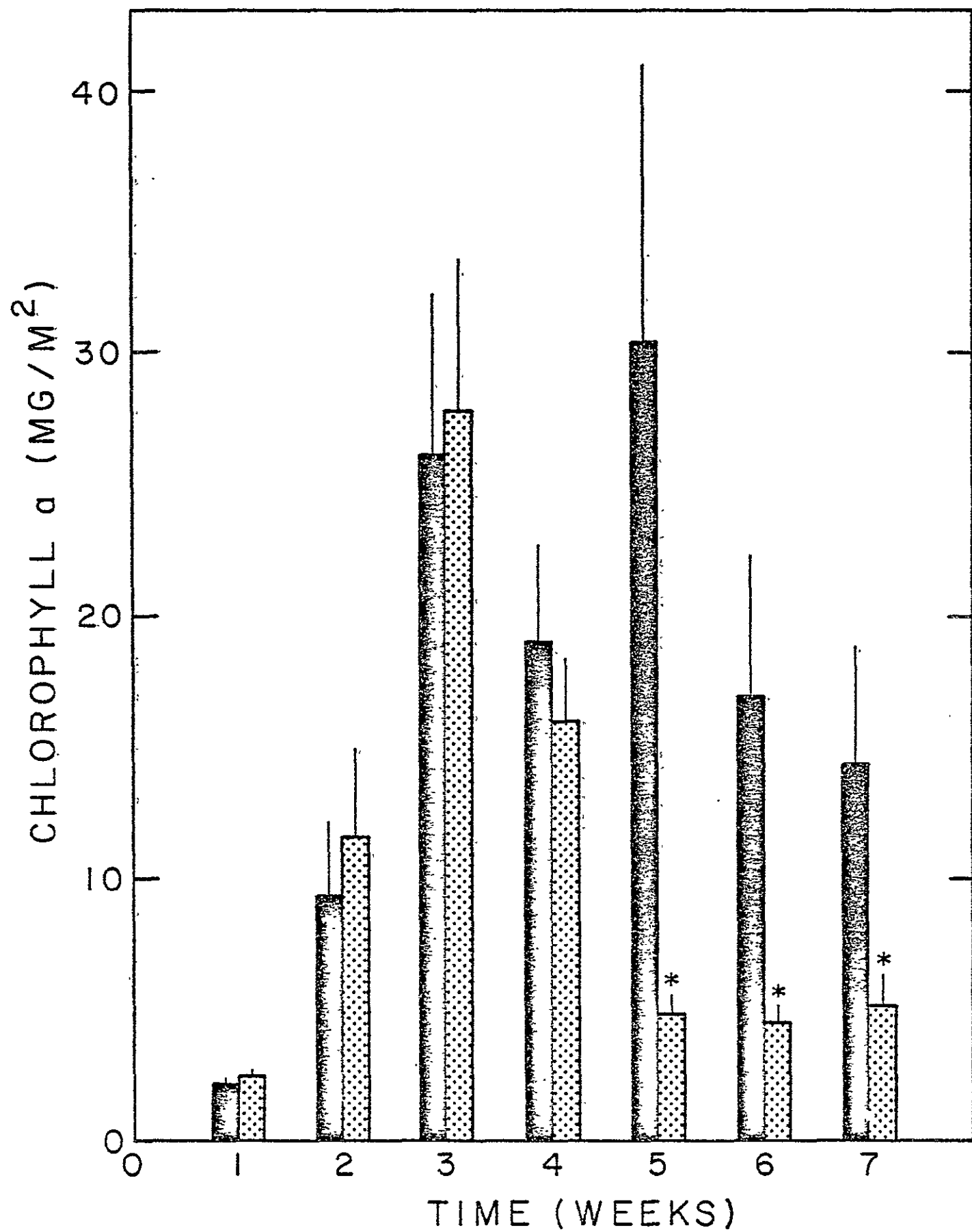
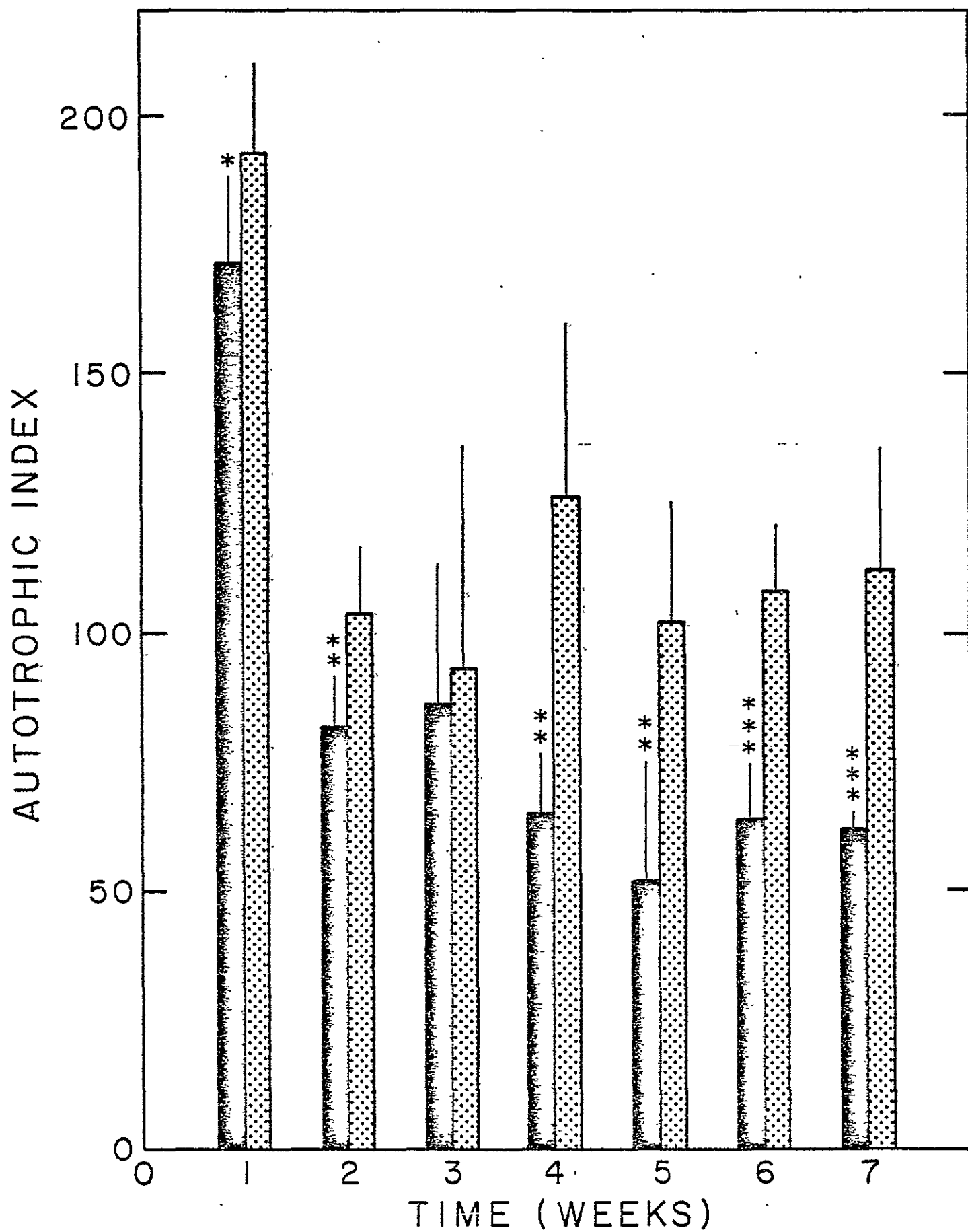
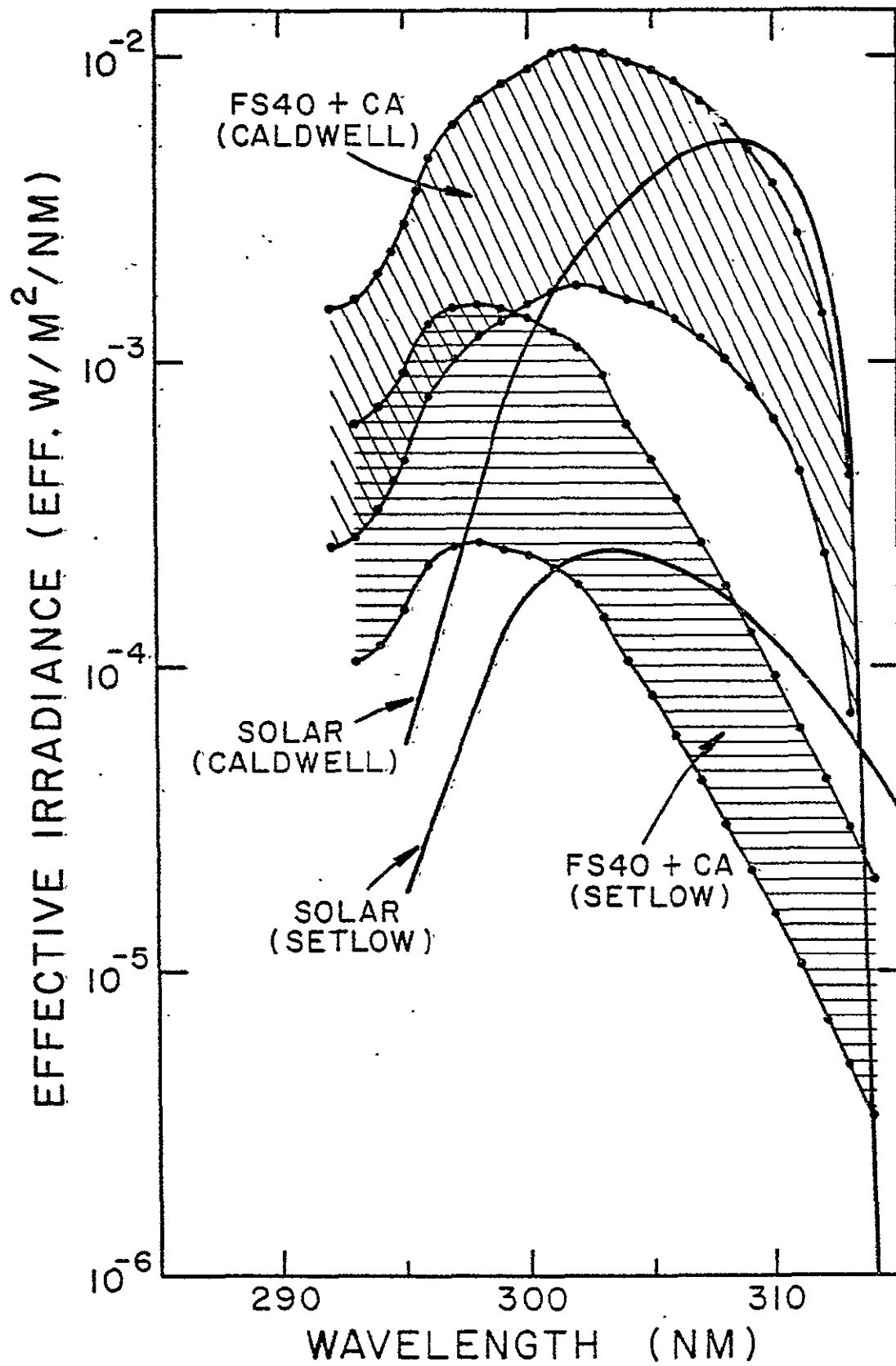


Figure 4. Autotrophic indices combined from samples taken at depths of 26 cm and 41 cm from the two flow-through chambers. Solid bars represent samples taken from the chamber receiving a surface irradiance of 1.0 W/m^2 (290-315 nm) and the shaded bars represent samples taken from the chamber receiving a surface irradiance of 1.4 W/m^2 . Error bars = ± 1 st. dev.; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.



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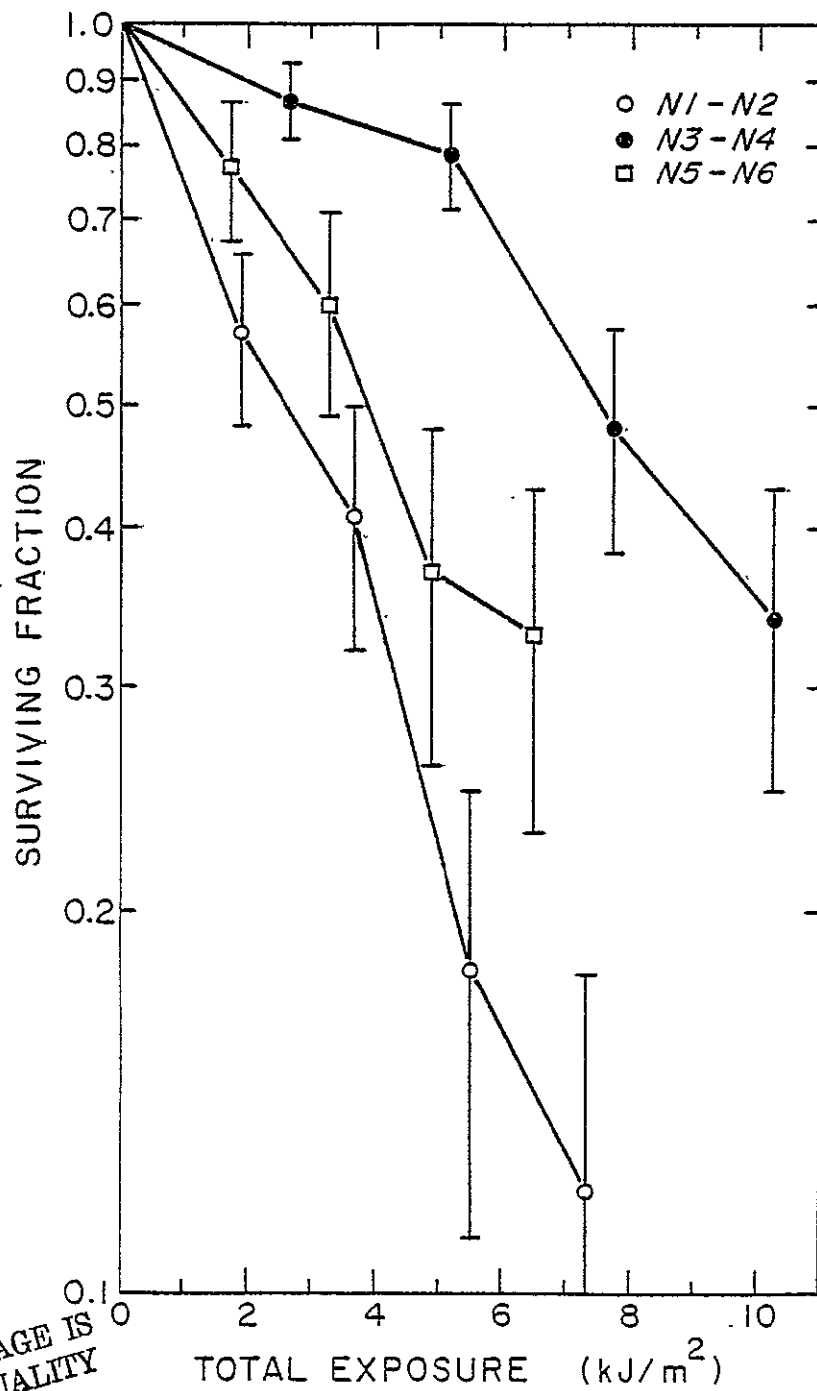
Figure 5. Practical range of biologically effective irradiance of sunlamp/filter system utilizing two different biological sensitivity factors: (1) Caldwell's (1968) generalized action spectrum, and (2) a DNA action spectrum compiled by Setlow (1974). Biologically weighted downward global irradiance spectra are illustrated, also.



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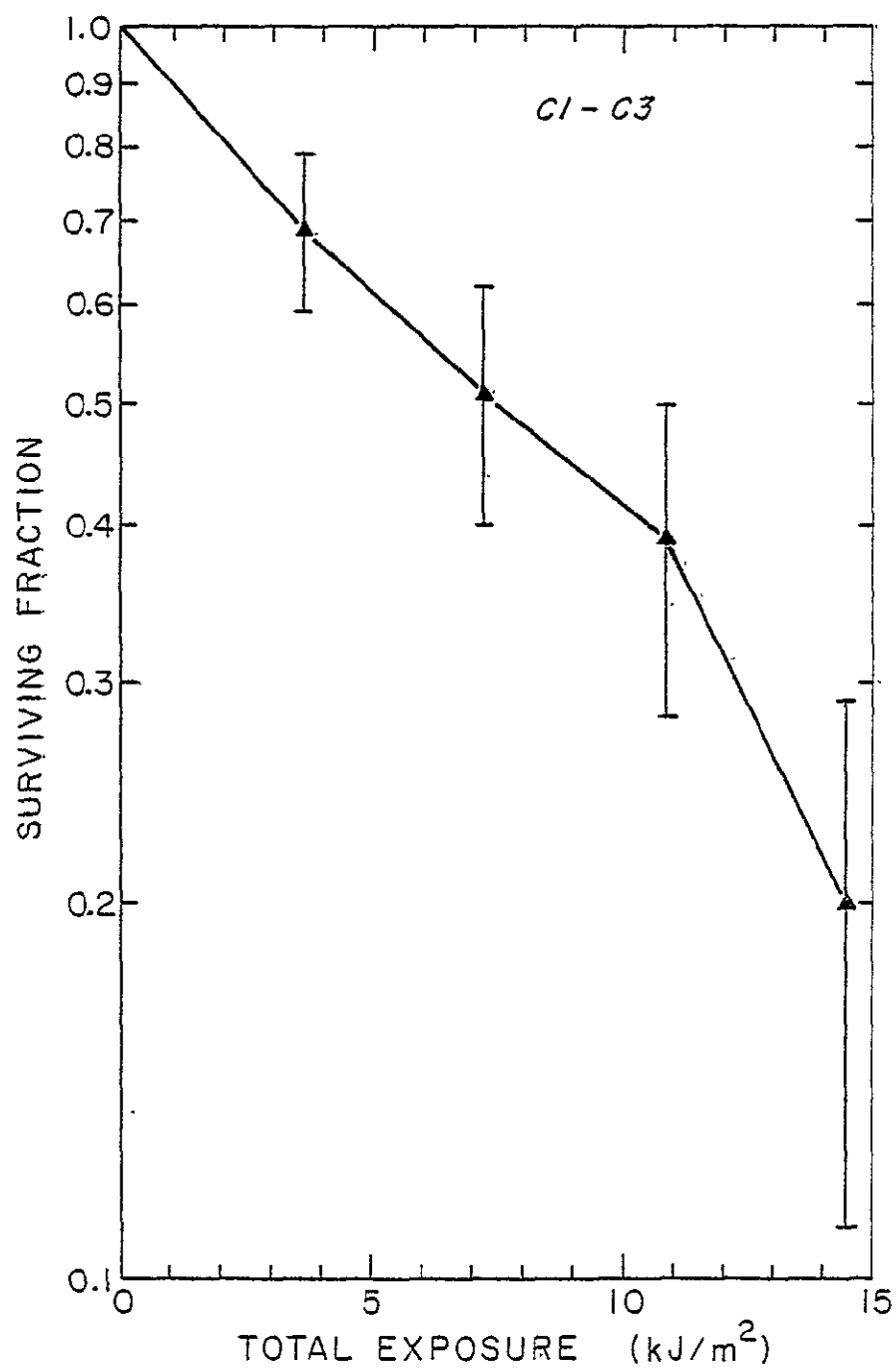
Figure 6. Survival curves for the naupliar stages of development of the marine copepod, *Acartia clausi*. A fluorescent sunlamp/cellulose triacetate filter system was used as the radiation source. Exposure rate in the 290–320 nm waveband, $1.43 \pm 0.08 \text{ W/m}^2$. 95% confidence intervals are shown.

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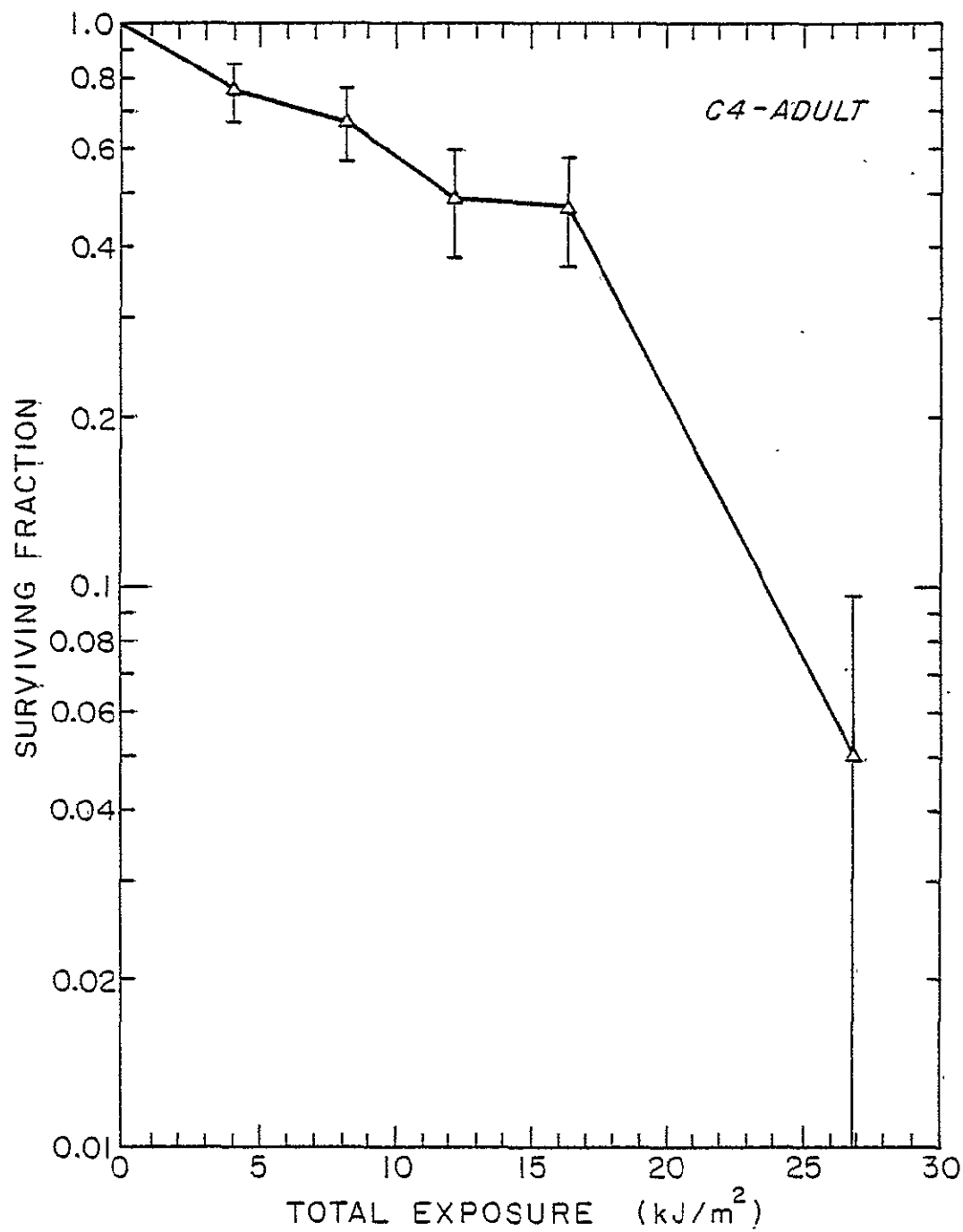
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Figure 7. Survival curve for the early copepodite age group (C1-C3) of *Acartia clausi*. A fluorescent sunlamp/cellulose triacetate filter system was used as the radiation source. Exposure rate in the 290-320 nm waveband, 1.34 W/m². 95% confidence intervals are shown.



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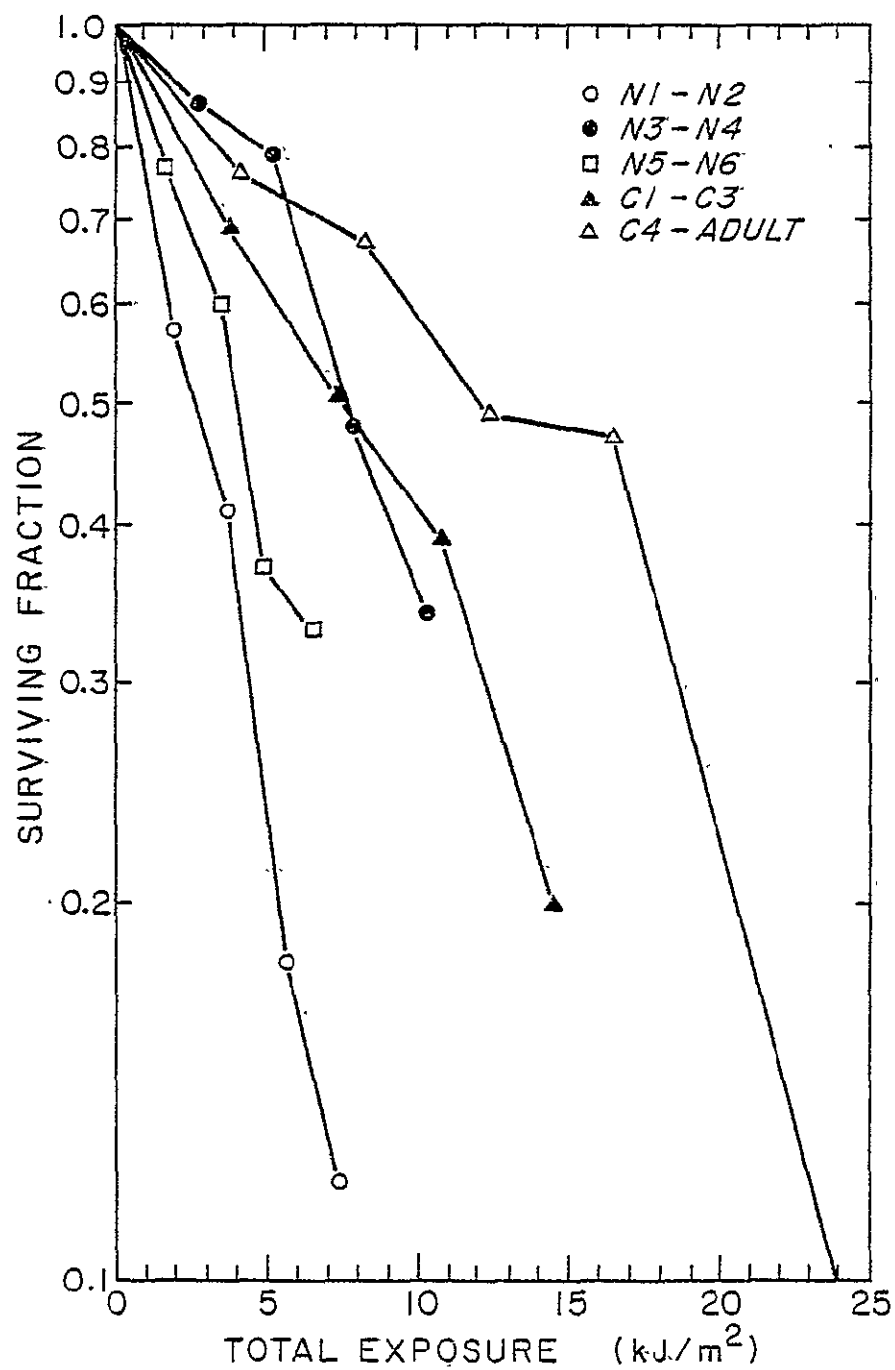
Figure 8. Survival curve for the late copepodite to adult age group of *Acartia clausi*. A fluorescent sunlamp/cellulose triacetate filter system was used as the radiation source. Exposure rate in the 290-320 nm waveband, 1.52 W/m². Each exposure group contained fifteen males and fifteen females. 95% confidence intervals are shown.



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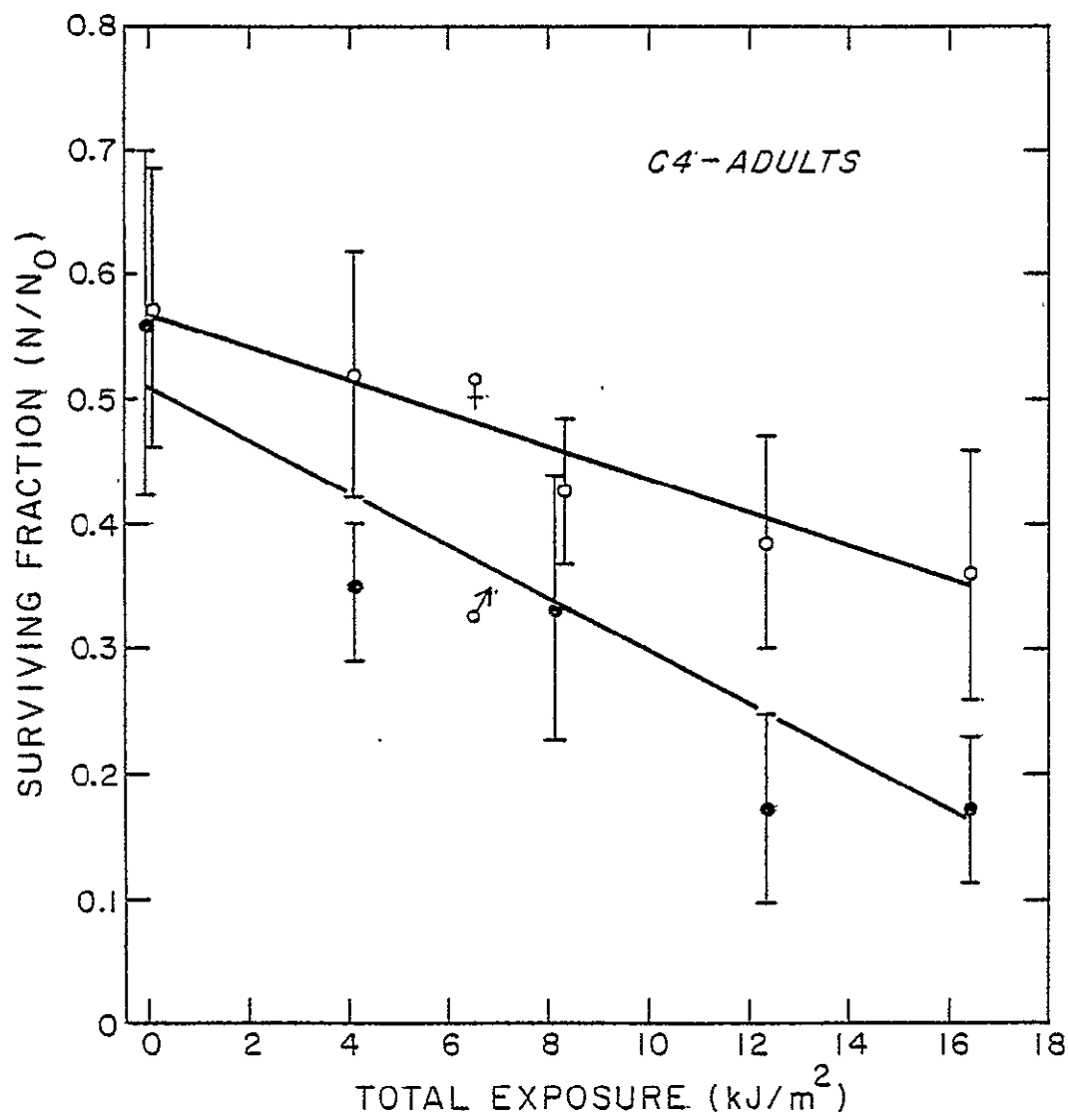
Figure 9. Survival curves for five different phases of development of the marine copepod, *Acartia clausi*. A fluorescent sunlamp/cellulose triacetate filter system was used as the radiation source. Exposure rate in the 290–320 nm waveband, $1.43 \pm 0.09 \text{ W/m}^2$.

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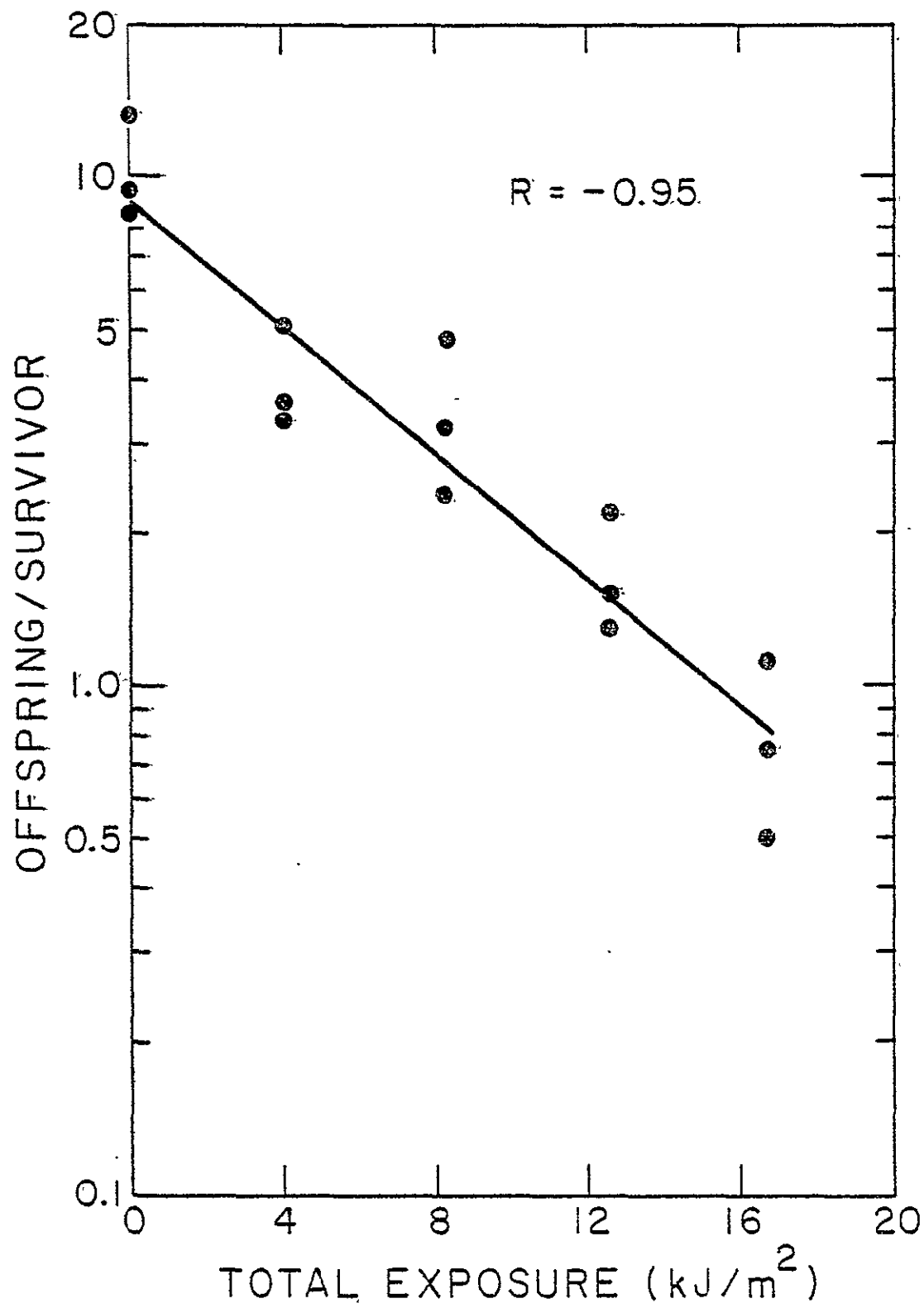
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Figure 10. Surviving fraction of males and females after exposure during the late copepodite to adult age group. Regression intercepts are not significantly different ($p > 0.05$). Slopes of the regressions (males = -0.023), female = -0.014) differ significantly ($p < 0.05$). Mean and standard deviation of original data are shown. Exposure in the 290–320 nm waveband, 1.52 W/m^2 .



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Figure 11. Relationship between the number of offspring produced per survivor of the irradiated C4-Adult age group and total dose as fitted by a least squares regression.



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